



# HOKKAIDO UNIVERSITY

|                  |                                                                                                                                                                                                                                   |
|------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Title            | Inhibition of algal bromophenols and their related phenols against glucose 6-phosphate dehydrogenase                                                                                                                              |
| Author(s)        | Mikami, Daisuke; Kurihara, Hideyuki; Ono, Momoka et al.                                                                                                                                                                           |
| Citation         | Fitoterapia, 108, 20-25<br><a href="https://doi.org/10.1016/j.fitote.2015.11.002">https://doi.org/10.1016/j.fitote.2015.11.002</a>                                                                                                |
| Issue Date       | 2016-01                                                                                                                                                                                                                           |
| Doc URL          | <a href="https://hdl.handle.net/2115/63940">https://hdl.handle.net/2115/63940</a>                                                                                                                                                 |
| Rights           | © 2016, Elsevier. Licensed under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International <a href="http://creativecommons.org/licenses/by-nc-nd/4.0/">http://creativecommons.org/licenses/by-nc-nd/4.0/</a> |
| Rights(URL)      | <a href="https://creativecommons.org/licenses/by-nc-nd/4.0/">https://creativecommons.org/licenses/by-nc-nd/4.0/</a>                                                                                                               |
| Type             | journal article                                                                                                                                                                                                                   |
| File Information | Kurihara.pdf                                                                                                                                                                                                                      |



**Title**

Inhibition of algal bromophenols and their related phenols against glucose 6-phosphate dehydrogenase

**Author names and affiliations**

Daisuke Mikami<sup>a</sup>, Hideyuki Kurihara<sup>b,\*</sup>, Momoka Ono<sup>b</sup>, Sang Moo Kim<sup>c</sup>, Koretaro Takahashi<sup>b</sup>

<sup>a</sup> Graduate School of Life Science, Faculty of Advanced Life Science, Hokkaido University, Kita-ku, Sapporo 001-0021, Japan.

<sup>b</sup> Faculty and Graduate School of Fisheries Sciences, Hokkaido University, Minato, Hakodate, Hokkaido 041-8611, Japan.

<sup>c</sup> Department of Marine Food Science and Technology, Gangneung-Wonju National University, Gangneung, Gangwon-do 210-702, Korea.

**Corresponding author**

Tel & Fax: +81 138 40 5561

E-mail: [kuri@fish.hokudai.ac.jp](mailto:kuri@fish.hokudai.ac.jp).

## Key words

Glucose 6-phosphate dehydrogenase, inhibition, bromophenol, polyphenol, alga

## Abstract

A novel bromophenol, *n*-butyl 2,3-dibromo-4,5-dihydroxybenzyl ether, and known bromophenols were isolated from Rhodomelaceae algae as glucose 6-phosphate dehydrogenase (G6PD) inhibitors. Among them, bromophenol dimers showed stronger inhibitory activity against *Leuconostoc mesenteroides* and *Saccharomyces cerevisiae* G6PDs than the corresponding monomers. The dibenzyl ether-type dimers had lower IC<sub>50</sub> values than the diarylmethane-type dimers against *L. mesenteroides* G6PD among the bromophenols examined. In contrast, the inhibitory activities of diarylmethane-type dimers against *S. cerevisiae* G6PD were stronger than those of dibenzyl ether-type dimers. Especially, 3-bromo-2-(2,3-dibromo-4,5-dihydroxybenzyl)-4,5-dihydroxybenzyl methyl ether selectively inhibited *S. cerevisiae* G6PD compared to *L. mesenteroides* G6PD.

## Abbreviations

G6PD, glucose 6-phosphate dehydrogenase; DHEA, dehydroepiandrosterone; *Lm*G6PD, *Leuconostoc mesenteroides* G6PD; *Sc*G6PD, *Saccharomyces cerevisiae* G6PD; 1-methoxy PMS, 1-methoxy-5-methylphenazium methylsulfate; ADAW, air-dried algal weight; RP, reversed phase.

## 1. Introduction

Glucose 6-phosphate dehydrogenase (G6PD, EC 1.1.1.49) is the first step and rate-determining enzyme in pentose phosphate pathway. G6PD catalyzes formation of reduced nicotinamide adenine dinucleotide phosphate (NADPH) and 6-phosphogluconolactone, a precursor of nucleotides. NADPH is an essential biological reductant for biosynthesis of fatty acid and cholesterol, reduced glutathione [1]. Lipogenic rate [2] and serum lipoprotein level [3] are decreased in G6PD-deficient patients. Dehydroepiandrosterone (DHEA), a specific mammalian G6PD inhibitor [4], leads to body weight loss of mice [5]. These studies suggest that G6PD is a potential therapeutic target of obesity. G6PD is also an important enzyme for cancer. Various cancer cells markedly elevate G6PD activity [6-9] because they require huge energy and nucleotide for their rapid growth. The rats ingested G6PD-overexpressed cells become carcinoma [10]. In contrast, G6PD-knockdown carcinoma cells show slow growth and increment of apoptosis [11]. In addition, G6PD inhibitors are possible to have anti-malarial and anti-trypanocidal activity [12, 13].

Although G6PD inhibitors are expected to possess promising effects, there are a few reports on G6PD inhibitors such as DHEA and its derivatives [4, 14], 6-aminonicotinamide [15], and catechin gallates [16]. In recent studies, small molecules were identified to be candidates of G6PD inhibitors [17-19]. Unfortunately, these compounds required high oral dosage [20] or interacted with proteins nonspecifically [16-18].

Marine algae produce various bioactive compounds such as terpenoids [21], polyphenols [22] and halogenated compounds [23-26]. Red algae, particularly Rhodomelaceae family, are rich sources of bromophenols. Bromophenols show various

beneficial functions, such as enzyme inhibition [27-35], anti-oxidative [35-37], anti-inflammatory [38], cell protection [39], anti-tumor [40], anti-diabetic activity [41, 42]. In the present study, we isolated a novel bromophenol **3**, along with known bromophenols **1**, **2**, **4-11**, and investigated inhibitory activity of the bromophenols and related phenolic compounds against prokaryotic *Leuconostoc mesenteroides* and eukaryotic *Saccharomyces cerevisiae* G6PDs.

## **2. Materials and Methods**

### *2.1. General*

*Leuconostoc mesenteroides* G6PD (EC 1.1.1.49) (*Lm*G6PD) and *Saccharomyces cerevisiae* G6PD (*Sc*G6PD) were purchased from Sigma-Aldrich (St. Louis, MO, USA). WST-1 and 1-methoxy-5-methylphenazium methylsulfate (1-methoxy PMS) were from Dojindo Laboratories (Mashiki, Kumamoto, Japan). Oxidized nicotinamide adenine dinucleotide phosphate (NADP<sup>+</sup>) was from Oriental Yeast Industries (Tokyo, Japan). Glucose 6-phosphate (G6P) and compounds **12-15** and **20** were purchased from Wako Pure Chemicals (Tokyo, Japan). Epigallocatechin gallate was from Cayman Chemical Company (Ann Arbor, MI, USA). Compounds **16-19**, and **21-26** were from Tokyo Chemical Industry (Tokyo, Japan). Compound **27** was from Avanti Polar Lipids, Inc. (Alabaster, USA). Column chromatography was performed with silica gel (Wakogel C-100, Wako Pure Chemicals, Osaka, Japan), reversed phase (RP) silica gel (Cosmosil 140C<sub>18</sub>-OPN, Nacalai Tesque, Kyoto, Japan), or Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). Thin layer chromatography (TLC)

was done using a glass plate precoated with silica gel 60 F<sub>254</sub> and RP-18 (Merck, Darmstadt, Germany), and spots were detected under UV light and visualized by spraying both 5% sulfuric acid and 1% potassium ferricyanide-1% ferric chloride reagents. High performance liquid chromatography (HPLC) was carried out using SHIMADZU LC-10AT<sub>VP</sub> apparatus (Kyoto, Japan) equipped with a diode array detector SHIMADZU SPD-M10A<sub>VP</sub> and an RP HPLC column (Mightysil RP18, Kanto Chemical, Co., Inc., Tokyo, Japan). NMR spectra were recorded in acetone-*d*<sub>6</sub> on a Bruker AMX-500 (Karlsruhe, Germany) spectrometer at 500 MHz for proton and 125 MHz for carbon chemical shifts. Field desorption-MS (FD-MS) spectra were recorded on a JEOL JMS-T100GCV spectrometer (Tokyo, Japan).

## 2.2. Algal materials

The algae *Odonthalia corymbifera*, *Neorhodomela aculeata* and *Symphycladia latiuscula* were collected at Hakodate in Japan in 2012 and 2013. They were identified by Prof. Hajime Yasui, Faculty of Fisheries Sciences, Hokkaido University. Algal samples were immediately brought to our laboratory. Voucher specimens of algae are deposited in our laboratory.

## 2.3. Extraction and isolation of algal bromophenols

### 2.3.1. Extraction

Collected algae were washed with tap water, cut into small pieces, and then extracted with methanol for 2 or 3 days. Methanol was evaporated under reduced

pressure with rotary evaporator (<35 °C). Residues were suspended in water and partitioned with *n*-hexane and then ethyl acetate. Bromophenols were separated into ethyl acetate-soluble fraction. Additionally, *N. aculeate* was also extracted with acetone. The extract was separated into ethyl acetate-soluble fraction in the same way.

### 2.3.2. Isolation from the methanol extract of *N. aculeate*

The ethyl acetate-soluble fraction (9.549 g) from the methanol extract of *N. aculeate* (1740 g, air-dried algal weight, ADAW) was chromatographed on silica gel to yield seven fractions. Fraction 4 (1.304 g) eluted with toluene/ethyl acetate (6:4, v/v) was further purified using RP column chromatography eluted with 70% aqueous methanol. Final purification was performed by RP HPLC eluted with 70% aqueous methanol containing 0.1% formic acid to obtain compounds **11** (112 mg, 0.00701% of ADAW) and **7** (8.7 mg, 0.00050% of ADAW). Fraction 5 (1945 mg) eluted with toluene/ethyl acetate (2:8, v/v) was further purified using RP column chromatography eluted with 40% aqueous methanol and preparative TLC developed with toluene/ethyl acetate/acetic acid (2:1:1, v/v/v). A part of this fraction (70 mg) was finally purified by RP HPLC eluted with 40% aqueous methanol containing 0.1% formic acid to give compound **1** (17.9 mg, 0.00897% of ADAW).

### 2.3.3. Isolation from the acetone extract of *N. aculeate*

The ethyl acetate-soluble fraction (5.547 g) from the acetone extract of *N. aculeata* (2580 g, ADAW) was chromatographed on silica gel to yield eight fractions. Fraction 3 (409 mg) eluted with toluene/ethyl acetate (8:2, v/v) was purified by RP

column chromatography eluted with 50% aqueous acetone to obtain compound **4** (76 mg, 0.029% of ADAW). Fraction 4 (137 mg) eluted with toluene/ethyl acetate (6:4, v/v) was purified by RP column chromatography eluted with 30% aqueous methanol to give compound **5** (26 mg, 0.010% of ADAW).

#### 2.3.4. Isolation from the methanol extract of *O. corymbifera*

The ethyl acetate-soluble fraction (9.992 g) from the methanol extract of *O. corymbifera* (630 g, ADAW) was chromatographed on silica gel to yield six fractions. Fraction 3 eluted with toluene/ethyl acetate (8:2, v/v) was further purified using RP column chromatography eluted with 60% aqueous methanol to obtain compounds **2** (1994 mg, 0.3165% of ADAW) and **3** (49 mg, 0.078% of ADAW). Compounds **8** and **10** were isolated according to the method of previous report [29].

#### 2.3.5. Isolation from the methanol extract of *S. latiuscula*

The ethyl acetate-soluble fraction (2.188 g) from the methanol extract of *S. latiuscula* (552 g, ADAW) was chromatographed on silica gel to yield eight fractions. Fraction 2 (516 mg) eluted with toluene/ethyl acetate (9:1, v/v) was further purified using RP column chromatography eluted with 50% aqueous acetone and preparative silica gel TLC developed with toluene/ethyl acetate/acetic acid (6:3:1, v/v/v). Final purification was done by RP HPLC to give compound **6** (6.1 mg, 0.0011% of ADAW) eluted with 70% aqueous methanol. Fraction 4 (797 mg) eluted with toluene/ethyl acetate (6:4, v/v) was purified using RP column chromatography eluted with 60% aqueous methanol to afford the fraction which contains bromophenols (343 mg). The

fraction was further purified by Sephadex LH-20 column chromatography eluted with methanol to give a bromophenol fraction (242 mg). Final purification was done by RP HPLC eluted with 85% aqueous methanol to obtain compound **9** (36 mg, 0.0058% of ADAW).

#### 2.4. Spectral data for novel compound **3**

Compound **3**: NMR  $\delta_H$  7.08 (1H, s, H-6), 4.43 (1H, s, H-7), 3.51 (2H, t, J = 6.4 Hz, H-1'), 1.57 (2H, tt, J = 7.4, 6.4 Hz, H-2'), 1.41 (2H, sextet, J = 7.4 Hz, H-3'), 0.91 (3H, t, J = 7.4 Hz, H-4');  $\delta_C$  131.9 (C-1), 114.3 (C-2), 113.6 (C-3), 144.3 or 145.5 (C-4), 144.3 or 145.5 (C-5), 115.3 (C-6), 73.3 (C-7), 70.9 (C-1'), 32.6 (C-2'), 20.0 (C-3'), 14.1 (C-4'); FD-MS (relative intensity)  $m/z$  352  $[M]^+$  (52), 354  $[M+2]^+$  (100), 356  $[M+4]^+$  (50); HR-FD-MS  $m/z$  351.95049  $[M]^+$  (calculated 351.94966 for  $C_{12}H_{16}O_2^{79}Br_2$ ).

#### 2.5. G6PD inhibitory assay

*Lm*G6PD inhibitory assay was carried out according to the method of Mikami et al. [29]. In brief, reaction mixture consisted of 1 M Tris-HCl buffer (pH 7.8, 22  $\mu$ L) containing 20 mM  $MgCl_2$ , 30 mM G6P (20  $\mu$ L), 10 mM  $NADP^+$  (6  $\mu$ L), distilled water (132  $\mu$ L) and test material (2  $\mu$ L) dissolved in dimethyl sulfoxide (DMSO). Enzyme reaction was initiated by adding 0.035 U/mL G6PD solution (20  $\mu$ L) to the reaction mixture, and incubated at 25 °C for 20 min. To terminate the enzyme reaction, saturated NaCl aqueous solution (1 mL) containing WST-1 (10  $\mu$ g) and 1-methoxy PMS (10  $\mu$ g) was added into the reaction mixture. The absorbance of resulting solution was measured

at 438 nm. ScG6PD assay was carried out according to the slightly modified method of Shin et al. [16]. Reaction mixture consisted of 1 M Tris-HCl buffer (pH 7.5, 25  $\mu$ L) containing 1.5 M NaCl and 60 mM MgCl<sub>2</sub>, 30 mM G6P (16.7  $\mu$ L), 10 mM NADP<sup>+</sup> (25  $\mu$ L), distilled water (130.8  $\mu$ L) and test materials (2.5  $\mu$ L) dissolved in DMSO. Enzyme reaction was initiated by adding 0.020 U/mL G6PD solution (50  $\mu$ L) to the reaction mixture, and incubated at 30 °C for 15 min. To terminate the enzyme reaction, saturated NaCl aqueous solution (1 mL) containing WST-1 (10  $\mu$ g) and 1-methoxy PMS (10  $\mu$ g) was added into the reaction mixture and the absorbance of resulting solution was measured at 438 nm.

### 3. Results and Discussion

#### 3.1. Structural determination of bromophenols

Compound **3** was isolated as yellow oil. It was confirmed to contain two bromine atoms from the molecular ion cluster at  $m/z$  352/354/356 (1:2:1). Molecular formula of the compound was determined by HR-FD-MS analysis to be C<sub>12</sub>H<sub>16</sub>O<sub>2</sub><sup>79</sup>Br<sub>2</sub>. The <sup>1</sup>H-NMR spectrum showed five aliphatic proton signals ( $\delta_H$  4.43, 3.51, 1.57, 1.41, 0.91) and an aromatic proton signal ( $\delta_H$  7.08). The COSY experiment (Fig. 2) revealed the presence of *O-n*-butyl group via spin couplings of triplet methyl proton at H-1' to triplet proton at H-4'. The <sup>13</sup>C-NMR spectrum contained five aliphatic and six aromatic carbon signals. In the HMBC experiment (Fig. 2), cross peaks were observed between benzyl proton signal at H-7 and carbon signals at C-1, C-2 and C-6. Thus an aromatic proton and a bromine atom were assigned to locate the *ortho* positions of the

oxymethylene group in the benzyloxy structure. Cross peaks were observed between the aromatic proton (H-6) and two oxygenated aromatic carbon signals (C-4, C-5). Therefore, compound **3** was confirmed to have an *ortho*-diphenol moiety. Based on these results, chemical structure of compound **3** was identified as *n*-butyl 2,3-dibromo-4,5-dihydroxy benzyl ether. This compound is a novel natural product like the marine-derived *n*-butylated compounds [43, 44], because *n*-butanol was not used as a solvent in the extraction and separation experiments. Structures of the other bromophenols **1**, **2**, **4-11** were determined with comparison of literature data [28, 29, 45-47], as shown in Fig. 1.

### 3.2. G6PD-inhibitory activity of the algal bromophenols

IC<sub>50</sub> values of bromophenols **1-11** on G6PD activity are shown in Table 1. The symmetric bromophenol dimers **8** and **9** of a dibenzyl ether type were potent inhibitors against *Lm*G6PD, while the symmetric bromophenol dimers **7**, **10** and **11** of a diarylmethane type were potent against *Sc*G6PD. Both types are different distance and orientation between two aromatic rings. This difference may be contributed to different accessibility to active sites of both G6PDs. Such different inhibitory activities between both types has been reported on aldose reductase inhibition [33]. The IC<sub>50</sub> value of compound **11** against *Sc*G6PD was most different, about 160-fold, from that against *Lm*G6PD, among the bromophenols examined. Hence, this compound would be used as a specific inhibitor for *Sc*G6PD. In addition, bromophenol monomer **3** showed stronger inhibitory activity against *Lm*G6PD, while bromophenol monomers **1** and **2** against *Sc*G6PD. This reason is also speculated as the difference in recognition sites of hydrophobic alkyl group of the side chain.

Bromophenol dimers **7-11** showed stronger inhibition than bromophenol monomers **1-3** and **6** against both *Lm*G6PD and *Sc*G6PD. In particular, symmetric dimers **8** and **9** showed considerably lower IC<sub>50</sub> values than the corresponding monomers **2** and **6**, respectively. The inhibition modes of bromophenol dimers **8** and **9** were similar to those of their corresponding monomers for yeast  $\alpha$ -glucosidase inhibition [28]. The inhibition modes of compounds **8** and **9** against G6PDs and  $\alpha$ -glucosidase might be similar because both enzymes are categorized to glucose-related enzymes.

### 3.3. Inhibitory activity of related phenolic compounds

IC<sub>50</sub> values of related phenols **12-27** (Fig. 3) on G6PD activity are shown in Table 2. Compounds **13**, **14**, **22-24**, **26** and **27** were moderate or weak inhibitors against both G6PDs. Catechol (**13**), pyrogallol (**14**) and symmetric 3,4-dihydroxyphenylpropanoid dimer **27** showed moderate G6PD inhibition, while phenol (**12**), phloroglucinol (**15**) and symmetric *p*-hydroxyphenylpropanoid dimer **26** showed no or weak inhibition. Thus *ortho*-diphenol moiety might be important for G6PD inhibition, like the previous report on galloyl moiety for G6PD inhibition [16].

Symmetric chlorinated phenol dimers **22-24** inhibited G6PD activity, while symmetric brominated phenol dimer **25** of a 2,2-diarylpropane type did not. Inhibition of the symmetric dimers against G6PDs may require a “simple bridge” between two aromatic rings rather than a “bulky bridge”.

Although 3,5-dibromo-4-hydroxybenzaldehyde inhibited *Lm*G6PD activity [29], the corresponding de-brominated 4-hydroxybenzaldehyde **20** did not inhibit G6PD activities. In the case of 4-hydroxybenzaldehydes, substitution of bromine might be

required to inhibit G6PD. On the other hand, halogenated phenol monomers **16-19** showed no inhibitory activity against both G6PD activities. The inhibitory activity of phenolic compounds against G6PD might depend on the substitution of halogen and other functional groups.

From the results in this study, G6PD inhibitor would require catechol or pyrogallol moieties and substitution by several bromine atoms. Furthermore, symmetric dimers of naturally occurring bromophenols enhance the inhibitory activity. It is unclear that the reasons why these bromophenols show potent inhibitory activity. In this study, naturally occurring bromophenols **7-11** purified from Rhodomelaceae algae showed strong or moderate inhibition against *Lm*G6PD and/or *Sc*G6PD, while related phenolic compounds **12-27** did no or weak inhibition. Based on these results, Rhodomelaceae algae are expected to be useful sources of G6PD inhibitors. Further investigation should be carried out to elucidate inhibition mechanisms using algal bromophenols and bromophenol analogs.

#### **4. Conclusion**

A novel bromophenol, *n*-butyl 2,3-dibromo-4,5-dihydroxybenzyl ether was isolated from Rhodomelaceae algae along with known bromophenols. In addition, the inhibitory activity of algal bromophenols were determined against prokaryotic G6PD (*Lm*G6PD) and eukaryotic G6PD (*Sc*G6PD). The symmetric bromophenol dimers of a dibenzyl ether type were potent inhibitors against *Lm*G6PD, while the symmetric bromophenol dimers of a diarylmethane type were potent against *Sc*G6PD. Compound **11** might be used as a specific inhibitor against *Sc*G6PD because its IC<sub>50</sub> values against

both G6PDs were considerably different.

### **Conflict of interest**

The authors declare no conflict of interest.

### **Acknowledgements**

We are grateful to Dr. Eri Fukushi and Mr. Yusuke Takata, GC-MS & NMR Laboratory, Faculty of Agriculture, Hokkaido University, for measurement of mass and NMR spectra. We also express our thanks to Professor Hajime Yasui, Faculty of Fisheries sciences, Hokkaido University, for identification of algae.

### **References**

- [1] Salati LM, Amir-Ahmady B. Dietary regulation of expression of glucose-6-phosphate dehydrogenase. *Annu. Rev. Nutr.* 2001; 21: 121-140.
- [2] Dessi S, Batteta B, Spano O, Pulisci D, Mulas MF, Muntoni S, Armeni M, Sanna C, Antonucci R, Pani P. Serum lipoprotein pattern as modified in G6PD-deficient children during haemolytic anaemia induced by fava bean ingestion. *Int. J. Exp. Pathol.* 1992; 73: 157-460.
- [3] Kreuzer G, Zajicek J. Cytologic diagnosis of mammary tumors from aspiration biopsy smears. 3. Studies on 200 carcinomas with false negative or doubtful cytologic reports. *Acta. Cytol.* 1972; 16: 249-252.

319

320 [4] Marks PA, Banks J. Inhibition of mammalian glucose-6-phosphate dehydrogenase  
321 by steroids. Proc. Natl. Acad. Sci. U.S.A. 1960; 46: 447-452.

322

323 [5] Yen TT, Allan AJ, Pearson DV, Acton JM, Greenberg MM. Prevention of obesity in  
324 A<sup>vy/a</sup> mice by dehydroepiandrosterone. Lipids 1977; 12: 409-413.

325

326 [6] Hughes EC. The effect of enzymes upon metabolism, storage, and release of  
327 carbohydrates in normal and abnormal endometria. Cancer 1976; 38: 487-502.

328

329 [7] Dutu R, Nedelea M, Veluda G, Burcuket, V. Cytoenzymologic investigations on  
330 carcinomas of the Cervix uteri. Acta. Cytol. 1980; 24: 160-166.

331

332 [8] Zampella EJ, Bradley EL Jr, Pretlow TG 2nd. Glucose-6-phosphate-dehydrogenase -  
333 a possible clinical indicator for prostatic-carcinoma. Cancer 1982; 49: 384-387.

334

335 [9] Dessi S, Batetta B, Cherchi R, Onnis R, Pisano M, Pani P. Hexose-monophosphate  
336 shunt enzymes in lung-tumors from normal and  
337 glucose-6-phosphate-dehydrogenase-deficient subjects. Oncology 1988; 45: 287-291.

338

339 [10] Kuo WY, Lin JY, Tang TK. Human glucose-6-phosphate dehydrogenase (G6PD)  
340 gene transforms NIH 3T3 cells and induces tumors in nude mice. Int. J. Cancer 2000;  
341 85: 857-864.

342

343 [11] Li D, Zhu Y, Tang Q, Lu H, Li H, Yang Y, Li Z, Tong S. A new G6PD knockdown

tumor-cell line with reduced proliferation and increased susceptibility to oxidative stress.  
Cancer Biother. Radiopharm. 2009; 24: 81-90.

[12] Cappadoro M, Giribaldi G, O'Brien E, Turrini F, Mannu F, Ulliers D, Simula G, Luzzatto L, Arese P. Early phagocytosis of glucose-6-phosphate dehydrogenase (G6PD)-deficient erythrocytes parasitized by *Plasmodium falciparum* may explain malaria protection in G6PD deficiency. Blood 1998; 92: 2527-2534.

[13] Preuss J, Maloney P, Peddibhotla S, Hedrick MP, Hershberger P, Gosalia P, Milewski M, Li YL, Sugarman E, Hood B, Suyama E, Nguyen K, Vasile S, Sergienko E. Mangravita-Novo A, Vicchiarelli M, McAnally D, Smith LH, Roth GP, Diwan J, Chung TDY, Jortzik E, Rahlfs S, Becker K, Pinkerton AB, Bode L. Discovery of a *Plasmodium falciparum* glucose-6-phosphate dehydrogenase 6-phosphogluconolactonase inhibitor (R,Z)-N-((1-ethylpyrrolidin-2-yl)methyl)-2-(2-fluorobenzylidene)-3-oxo-3,4-dihydro-2H-benzo[b][1,4] thiazine-6-carboxamide (ML276) that reduces parasite growth *in vitro*. J. Med. Chem. 2012; 55: 7262-7272.

[14] Hamilton NM, Dawson M, Fairweather EE, Hamilton SN, Hitchin JR, James DI, Jones SD, Jordan AM, Lyones AJ, Small HF, Thomson GJ, Waddell ID, Ogilvie DJ. Novel steroid inhibitors of glucose 6-phosphate dehydrogenase. J. Med. Chem. 2012; 55: 4431-4445.

[15] Köhler E, Barrach HJ, Neubert D. Inhibition of NADP dependent oxidoreductases by the 6-aminonicotinamide analogue of NADP. FEBS Lett. 1970; 6: 225-228.

[16] Shin ES, Park J, Shin JM, Cho D, Cho SY, Shin DW, Ham M, Kim JB, Lee TR. Catechin gallates are NADP<sup>+</sup>-competitive inhibitors of glucose-6-phosphate dehydrogenase and other enzymes that employ NADP<sup>+</sup> as a coenzyme. Bioorg. Med. Chem. 2008; 16: 3580-3586.

[17] Preuss J, Hedrick M, Sergienko E, Pinkerton A, Mangravita-Novo A, Smith L, Marx C, Fischer E, Jortzik E, Rahlfs S, Becker K, Bode L. High-throughput screening for small-molecule inhibitors of *Plasmodium falciparum* glucose-6-phosphate dehydrogenase 6-phosphogluconolactonase. J. Biomol. Screen. 2012; 17: 738-751.

[18] Preuss J, Richardson AD, Pinkerton A, Hedrick M, Sergienko E, Rahlfs S, Becker K, Bode L. Identification and characterization of novel human glucose-6-phosphate dehydrogenase inhibitors. J. Biomol. Screen. 2013; 18: 286-297.

[19] Beydemir S, Gulcin I, Kufrevioglu OI, Ciftci M. Glucose 6-phosphate dehydrogenase: *in vitro* and *in vivo* effects of dantrolene sodium. Pol. J. Pharmacol. 2003; 55: 787-792.

[20] Lardy H, Marwah A, Marwah P. Transformations of DHEA and its metabolites by rat liver. Lipids 2002; 37, 1187-1191.

[21] Ochi M, Watanabe M, Miura I, Taniguchi M, Tokoroyama T. Amijiol, isoamijiol, and 14-deoxyamijiol, 3 new diterpenoids from the brown seaweed *Dictyota linearis*. Chem. Lett. 1988; 9: 1229-1232.

- [22] Glombitza KW, Lentz G. Antibiotics from algae. 28. Cleavage of high molecular phlorotannin derivatives from the brown alga *Fucus vesiculosus* L. Tetrahedron 1981; 37: 3861-3866.
- [23] Ohta K, Takagi M. Halogenated sesquiterpenes from the marine red alga *Marginisporum aberrans*. Phytochemistry 1977; 16: 1062-1063.
- [24] Takahashi Y, Suzuki M, Abe T, Masuda M. Anhydroaplysiadiol from *Laurencia japonensis*. Phytochemistry 1988; 48: 987-990.
- [25] Lin AS, Stout EP, Prudhomme J, Le Roch K, Fairchild CR, Franzblau SG, Aalbersberg W, Hay ME, Kubanek J. Bioactive bromophycolides R–U from the Fijian red alga *Callophycus serratus*. J. Nat. Prod. 2010; 73: 275–278.
- [26] Wang BG, Gloer JB, Ji NY, Zhao JC. Halogenated organic molecules of Rhodomelaceae origin: chemistry and biology. Chem. Rev. 2013; 113: 3632-3685.
- [27] Kurihara H, Mitani T, Kawabata J, Takahashi K. Two new bromophenols from the red alga *Odonthalia corymbifera*. J. Nat. Prod. 1999; 62: 882-884.
- [28] Kurihara H, Mitani T, Kawabata J, Takahashi K. Inhibitory potencies of bromophenols from Rhodomelaceae algae against  $\alpha$ -glucosidase activity. Fish. Sci. 1999; 65: 300–303.
- [29] Mikami D, Kurihara H, Kim SM, Takahashi K. Red algal bromophenols as glucose

6-phosphate dehydrogenase inhibitors. Mar. Drugs 2013; 11: 4050-4057.

[30] Kim KY, Choi KS, Kurihara H, Kim SM.  $\beta$ -Glucuronidase inhibitory activity purified from *Grateloupia elliptica*. Food Sci. Biotechnol. 2008; 17: 1110-1114.

[31] Kim KY, Nam KA, Kurihara H, Kim SM. Potent  $\alpha$ -Glucosidase inhibitors purified from the red alga *Grateloupia elliptica*. Phytochemistry 2008; 69: 2820-2825.

[32] Kim KY, Nguyen TH, Kurihara H, Kim SM.  $\alpha$ -Glucosidase inhibitory activity of bromophenol purified from the red alga *Polyopes lancifolia*. J. Food Sci. 2010; 75: H145-H150.

[33] Wang W, Okada Y, Shi H, Wang Y, Okuyama T. Structures and aldose reductase inhibitory effects of bromophenols from the red alga *Symphyocladia latiuscula*. J. Nat. Prod. 2005; 68: 620-622.

[34] Boztas M, Cetinkaya Y, Topal M, Gulcin I, Menzek A, Sahin E, Tanc M, Supuran CT. Synthesis and carbonic anhydrase isozymes I, II, IX, and XII inhibitory effects of dimethoxybromophenol derivatives incorporating cyclopropane moieties. J. Med. Chem. 2015; 58: 640-650.

[35] Oztaskin N, Cetinkaya Y, Taslimi P, Goksu S, Gulcin I. Antioxidant and acetylcholinesterase inhibition properties of novel bromophenol derivatives. Bioorg. Chem. 2015; 60: 49-57.

- [36] Li K, Li XM, Ji NY, Wang BG. Bromophenols from the marine red alga *Polysiphonia urceolata* with DPPH radical scavenging activity. J. Nat. Prod. 2008; 71: 28-30.
- [37] Balaydin HT, Gulcin I, Menzak A, Goksu S, Sahin E. Synthesis and antioxidant properties of diphenylmethane derivative bromophenols including a natural product. J. Enzym. Inhib. Med. Chem. 2010; 25: 685-695.
- [38] Wiemer DF, Idler DD, Fenical W. Vidalols A and B, new anti-inflammatory bromophenols from the Caribbean marine red alga *Vidalia obtusiloba*. Experientia 1991; 47: 851-853.
- [39] Olsen EK, Hansen E, Isaksson J, Andersen JH. Cellular antioxidant effect of four bromophenols from the red algae, *Vertebrata lanosa*. Mar. Drugs 2013; 11: 2769-2784.
- [40] Xu XL, Song FH, Wang SJ, Li S, Xiao F, Zhao JL, Yang YC, Shan SQ, Yang L, Shi JG. Dibenzyl bromophenols with diverse dimerization patterns from the brown alga *Leathesia nana*. J. Nat. Prod. 2004; 67: 1661-1666.
- [41] Shi DY, Xu F, He J, Li J, Fan X, Han LJ. Inhibition of bromophenols against PTP1B and anti-hyperglycemic effect of *Rhodomela confervoides* extract in diabetic rats. Chin. Sci. Bull. 2008; 53: 2476-2479.
- [42] Shi D, Guo S, Jiang B, Guo C, Wang T, Zhang L, Li J. HPN, a synthetic analogue

of bromophenol from red alga *Rhodomela confervoides*: synthesis and anti-diabetic effects in C57BL/KsJ-db/db mice. Mar. Drugs 2013; 11: 350-362.

[43] Hayat S, Atta-ur-Rhaman, Choudhary MI, Khan KM, Abbaskhan A. Two new cinnamic acid esters from marine brown alga *Spatoglossum variabile*. Chem. Pharm. Bull. 2002; 50: 1297-1299.

[44] Xu X, He F, Zhang X, Bao J, Qi S. New mycotoxins from marine-derived fungus *Aspergillus* sp. SCSGAF0093. Food Chem. Toxicol. 2014; 53: 46-51.

[45] Kurata K, Amiya T. A New Bromophenol from the Red Alga *Polysiphonia urceolata*. Bull. Chem. Soc. Jpn. 1980; 53: 2020-2022.

[46] Woo LWL, Bubert C, Sutcliffe OB, Smith A, Chander SK, Mahon MF, Purohit A, Reed MJ, Potter BVL. Dual aromatase-steroid sulfatase inhibitors. J. Med. Chem. 2007; 5: 3540-3560.

[47] Kurata K, Amiya T. Two new bromophenols from the red alga, *Rhodomela larix*. Chem. Lett. 1977; 6: 1435-1438.

**Table 1**

IC<sub>50</sub> values of algal bromophenols against *Leuconostoc mesenteroides* and *Saccharomyces cerevisiae* glucose 6-phosphate dehydrogenases.

| Compound                        | IC <sub>50</sub> <sup>a</sup> (μM) |                             |
|---------------------------------|------------------------------------|-----------------------------|
|                                 | <i>Lm</i> G6PD <sup>b</sup>        | <i>Sc</i> G6PD <sup>c</sup> |
| <b>1</b>                        | 218 ± 5.0                          | 40.4 ± 8.1                  |
| <b>2</b>                        | >1000                              | 572 ± 3                     |
| <b>3</b>                        | 321 ± 18                           | >1000                       |
| <b>4</b>                        | >1000                              | >1000                       |
| <b>5</b>                        | >1000                              | 50.2 ± 6.0%                 |
| <b>6</b>                        | 289 ± 32                           | 401 ± 89                    |
| <b>7</b>                        | 4.54 ± 0.82                        | 0.47 ± 0.03                 |
| <b>8</b>                        | 0.85 ± 0.10 <sup>d</sup>           | 3.23 ± 0.05                 |
| <b>9</b>                        | 0.97 ± 0.10                        | 0.95 ± 0.04                 |
| <b>10</b>                       | 4.01 ± 0.30 <sup>d</sup>           | 0.39 ± 0.23                 |
| <b>11</b>                       | 86.1 ± 5.0                         | 0.53 ± 0.18                 |
| <b>Epigallocatechin gallate</b> | 7.70 ± 0.14 <sup>d</sup>           | 2.55 ± 0.14                 |

<sup>a</sup> Mean ± SD (*n* = 3). Inhibition assay was carried out at the substrate G6P and NADP<sup>+</sup> concentrations of 3.0 and 0.3 mM for *Lm*G6PD, and 2.0 and 1.0 mM *Sc*G6PD, respectively; <sup>b</sup>*Leuconostoc mesenteroides* glucose 6-phosphate dehydrogenase; <sup>c</sup>*Saccharomyces cerevisiae* glucose 6-phosphate dehydrogenase; <sup>d</sup> Literature data [29].

**Table 2**

IC<sub>50</sub> values of phenolic compounds against *Leuconostoc mesenteroides* and *Saccharomyces cerevisiae* glucose 6-phosphate dehydrogenases.

| Compound  | IC <sub>50</sub> <sup>a</sup> (μM) |                             |
|-----------|------------------------------------|-----------------------------|
|           | <i>Lm</i> G6PD <sup>b</sup>        | <i>Sc</i> G6PD <sup>c</sup> |
| <b>12</b> | >1000                              | >1000                       |
| <b>13</b> | 282 ± 24                           | 354 ± 52                    |
| <b>14</b> | 15.9 ± 1.1                         | 12.4 ± 0.4                  |
| <b>15</b> | >1000                              | >1000                       |
| <b>16</b> | >1000                              | >1000                       |
| <b>17</b> | >1000                              | >1000                       |
| <b>18</b> | >1000                              | >1000                       |
| <b>19</b> | >1000                              | >1000                       |
| <b>20</b> | >1000                              | >1000                       |
| <b>21</b> | >1000                              | >1000                       |
| <b>22</b> | 282 ± 22                           | 122 ± 4                     |
| <b>23</b> | 39.2 ± 2.1                         | 4.18 ± 0.42                 |
| <b>24</b> | 136 ± 2.1                          | 13.7 ± 34                   |
| <b>25</b> | >1000                              | 200 ± 34                    |
| <b>26</b> | 678 ± 35                           | 154 ± 15                    |
| <b>27</b> | 40.4 ± 9.0                         | 14.8 ± 0.1                  |

<sup>a</sup> Mean ± SD (*n* = 3). Inhibition assay was carried out at the substrate G6P and NADP<sup>+</sup> concentrations of 3.0 and 0.3 mM for *Lm*G6PD, and 2.0 and 1.0 mM *Sc*G6PD, respectively; <sup>b</sup>*Leuconostoc mesenteroides* glucose 6-phosphate dehydrogenase; <sup>c</sup>*Saccharomyces cerevisiae* glucose 6-phosphate dehydrogenase.

507 Figure captions

508

509 **Fig. 1. The structures of algal bromophenols.**

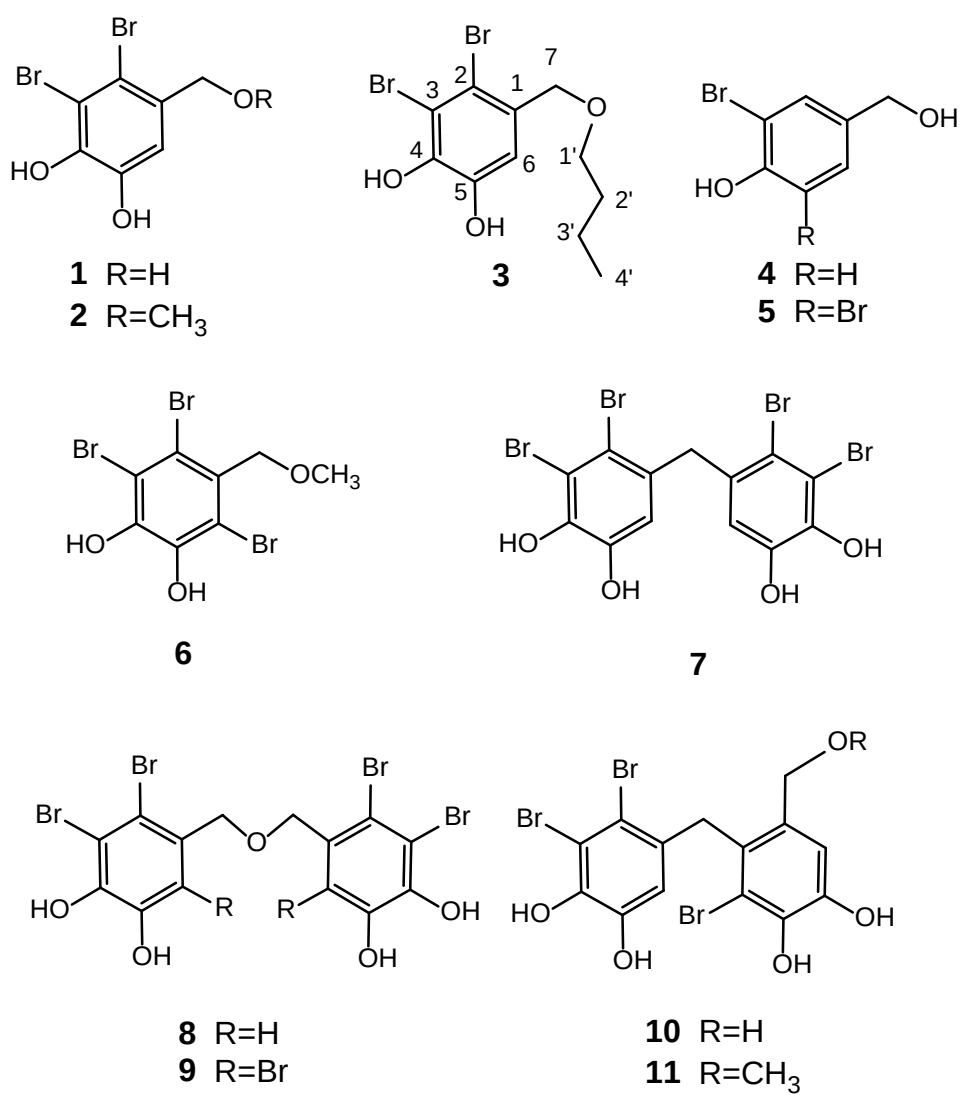
510

511 **Fig. 2.  $^1\text{H}$ - $^1\text{H}$  COSY and HMBC correlations of compound 3.**

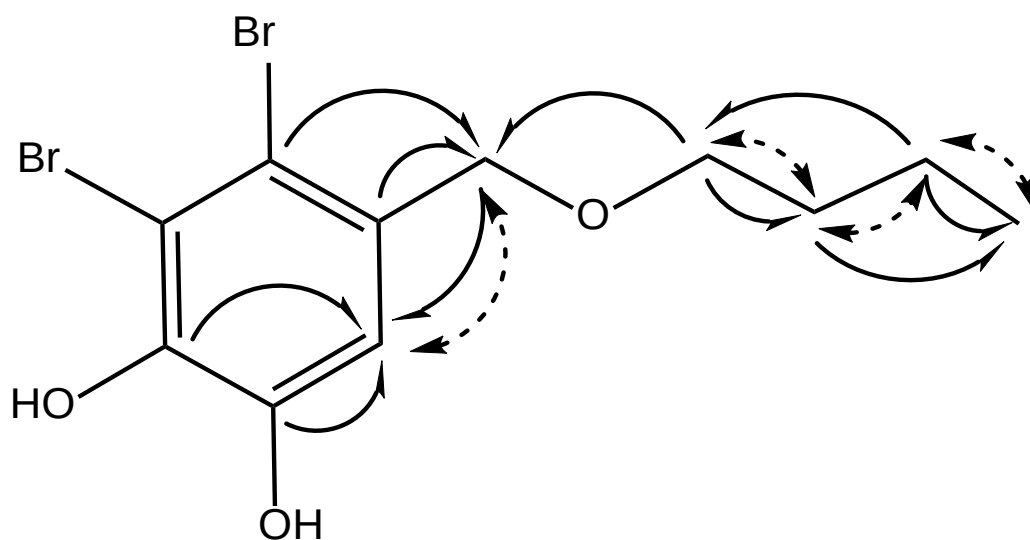
512  $^1\text{H}$ - $^1\text{H}$  COSY and HMBC correlations are indicated as dashed line arrows and solid  
513 half-headed arrows, respectively.

514

515 **Fig. 3. Phenolic compounds used for G6PD inhibitory assays.**

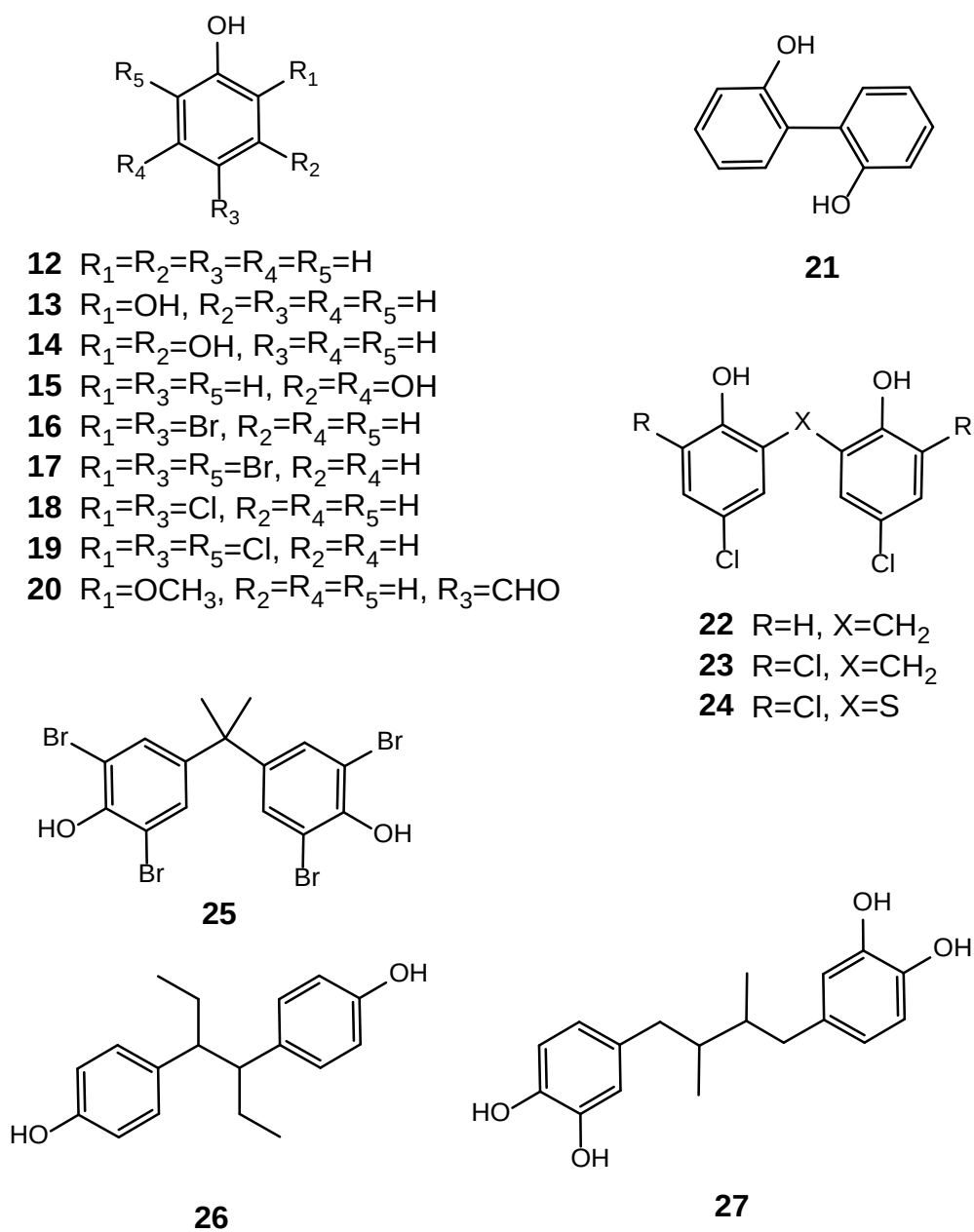


**Fig. 1. The structures of algal bromophenols.**



**Fig. 2. <sup>1</sup>H-<sup>1</sup>H COSY and HMBC correlations of compound 3.**

<sup>1</sup>H-<sup>1</sup>H COSY and HMBC correlations are indicated as dashed line arrows and solid half-headed arrows, respectively.



**Fig. 3. Phenolic compounds used for G6PD inhibitory assays.**